

# First ultrastructural observation of spermatozoa in a desmoscolecid nematode (Nematoda: Desmoscolecida: Desmoscolecidae)

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**Summary.** The spermatozoa from testis of the free-living marine nematode *Desmoscolex granulatus* (Desmoscolecida) were studied electron-microscopically. The spermatozoa are unpolarized cells covered by numerous filopodia. They contain the central lobated nucleus without a nuclear envelope. The spermatozoan cytoplasm includes mitochondria and fibrous bodies (FB). The spermatozoa of *D. granulatus* lack membranous organelles (MO) – a characteristic feature found in many nematode spermatozoa. The spermatozoon pattern, with the presence of FB never being associated with MO, unites *D. granulatus* with some chromadorids, desmodorids (Desmodoridae), monhysterids (Linhomoeidae) and tylenchomorphs (Tylenchoidea). This conclusion is supported by the filopodial nature of the sperm surface demonstrated by these taxa.

**Key words:** *Desmoscolex granulatus*, fibrous bodies, filopodia, membranous organelles, spermatogenesis.

Nematode spermatozoa represent an aberrant type of male gametes; they are characterised by the absence of an axoneme and an acrosome and have several unique features (Justine & Jamieson, 1999; Justine, 2002; Yushin & Malakhov, 2004, 2014). In the classification based on morphological and molecular data proposed by De Ley & Blaxter (2002), the phylum Nematoda is subdivided into two classes: Enoplea and Chromadorea. The structure and development of nematode sperm have been studied mainly for representatives of the extensive order Rhabditida belonging to Chromadorea (Justine & Jamieson, 1999; Justine, 2002). Most species studied within Rhabditida produce relatively uniform sperm of the ‘rhabditid pattern’ (Yushin & Malakhov, 2014). This type of nematode spermatozoon is seen as an amoeboid bipolar cell with an anterior pseudopod and posterior main cell body, which includes a condensed nucleus without a nuclear envelope, mitochondria and so-called ‘membranous organelles’ (MO), the unique organelles

characteristic of both the developing and mature sperm of most nematodes studied (Justine & Jamieson, 1999; Justine, 2002; Yushin & Malakhov, 2004, 2014). The MO are derived from the Golgi bodies and develop as a part of the complexes with paracrystalline fibrous bodies (FB) – another unique component of developing sperm. The prism-shaped FB are composed of densely packed parallel filaments consisting of the unique cytoskeleton protein MSP (‘major sperm protein’) (Justine & Jamieson, 1999; Justine, 2002; Chu & Shakes, 2013; Yushin *et al.*, 2016). The outlined basic pattern of sperm structure and development is characteristic for representatives of the class Chromadorea, especially for the well-studied order Rhabditida (Justine & Jamieson, 1999; Justine, 2002; Yushin & Malakhov, 2004, 2014; Zograf, 2014; Slos *et al.*, 2015). However, the nematode order Rhabditida and other orders of Chromadorea include taxa for which sperm development and structure have distinct deviations from the ‘rhabditid pattern’ (Justine & Jamieson, 1999; Yushin & Spiridonov, 2001;

Justine, 2002; Yushin & Malakhov, 2004, 2014). Developing male gametes have a large number of specific cytological characters that can be compared and analysed for in depth discussion of metazoan taxonomy and phylogeny (Baccetti, 1985; Jamieson *et al.*, 1995; Liana & Witalinski, 2005; Pitnick *et al.*, 2009; Levron *et al.*, 2010; Dallai *et al.*, 2016). It was hypothesised that the basic spermatozoon features also reflect position of nematode taxa on the nematode phylogenetic tree (Yushin & Malakhov, 2004, 2014).

Species of *Desmoscolex* and other Desmoscolecini are distinguished among marine nematode taxa by their peculiar appearance as well as by some unusual traits in their fine morphology and biology. The body cuticle consists of broad and convex main rings with thin and flexible inter-zones between them. The main rings are covered with so-called desmen composed of angular concretions (Fig. 1). The latter are not derived from secretions but comprise sticky clay mineral platelets and sand grains (Riemann & Riemann, 2010; Decraemer & Rho, 2014; personal data of A.V. Tchesunov). The compact head bears four pedunculated cephalic setae and large blister-like amphideal fovea, the amphids being covered with thin cuticle (personal data of A.V. Tchesunov). Other peculiar features are so-called phasmata (lateral circular pores on the end ring of the tail) and pigment spots at the level of the anterior intestine. Females have two opposed outstretched ovaries. In relation to reproductive biology, an unusual feature was mentioned for some desmoscolecids (Timm, 1970): females can carry their developing eggs attached to the body, either glued to the tail (*Tricoma*) or pressed to the ventral body side by elongate setae (*Desmoscolex*). Males have either two opposed testes (Tricominae) or only a single anterior outstretched testis (Desmoscolecinae).

The phylogenetic position of Desmoscolecida among Nematoda as well as the classification within the order is still under discussion (Decraemer & Rho, 2014). Hwang *et al.* (2009) provided nucleotide sequences of 18S rDNA for representatives of four desmoscolecid genera (*Desmoscolex*, *Greeffiella*, *Tricoma* and *Paratricoma*) and came to the conclusion that those genera form a clear clade that could be treated as sister group of Monhysterida (including Comesomatidae). New morphological data on male gametes may assist in resolving problems in nematode relationships, including the position of the order Desmoscolecida within the phylum. The fine structure of male gametes in Desmoscolecida has not been studied. In light microscopical

observations, the male spermatogenic cells are usually mentioned briefly as large spermatocytes and small globular spermatozoa (Timm, 1970).

The nematode species, *D. granulatus* Decraemer, 1975 belonging to the genus *Desmoscolex* Claparède, 1863 (Desmoscolecini, Desmoscolecidae), was chosen for the first ultrastructural study of the desmoscolecid spermatozoon to perform comparative analysis with spermatozoa of other nematodes. The ultrastructure of immature spermatozoa from the testes of *Desmoscolex* has been studied in details.

## MATERIAL AND METHODS

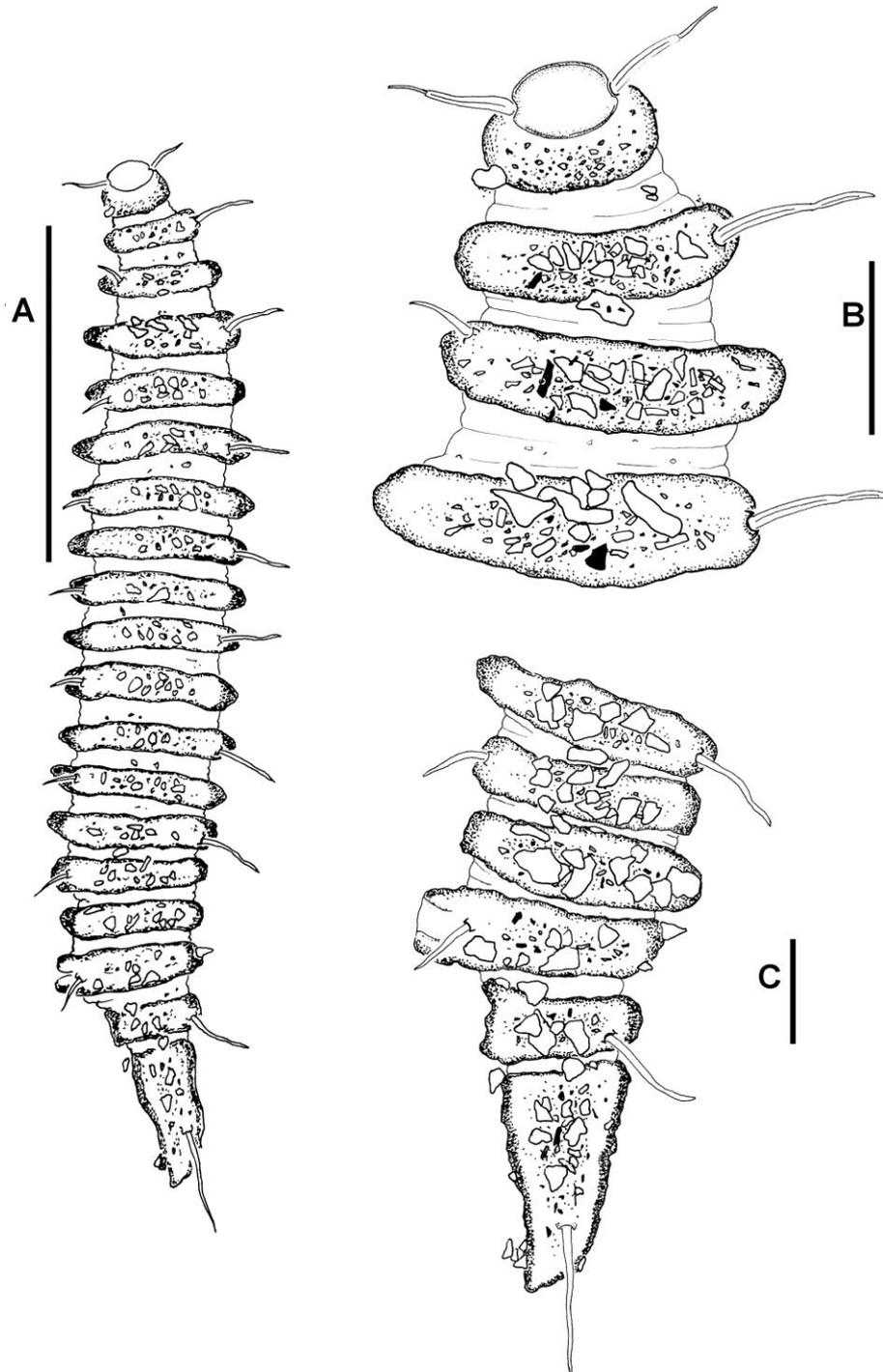
Samples were collected in the North Vietnam, Tien Yen Estuary (21°18'997" N; 107°36'075" E) in April 2015. Bottom sediments were fine silty sands, and water depths of 0.5 m. Benthic meiofauna was sampled using cores of 3.6 cm inner diameter (surface of 10 cm<sup>2</sup>). The samples were washed through 1 mm and 40 µm sieves.

Live nematodes belonging to the genus *Desmoscolex* were picked out from the samples under a stereoscopic microscope. For light microscopy, nematodes were fixed with 4% paraformaldehyde and transferred to glycerin using the Seinhorst's (1959) rapid method as modified by De Grisse (1969), and mounted on permanent slides. Difficulties in identification of desmoscolecid species required detailed description of specimens collected for this study. The male used for TEM had the same general morphology as the female.

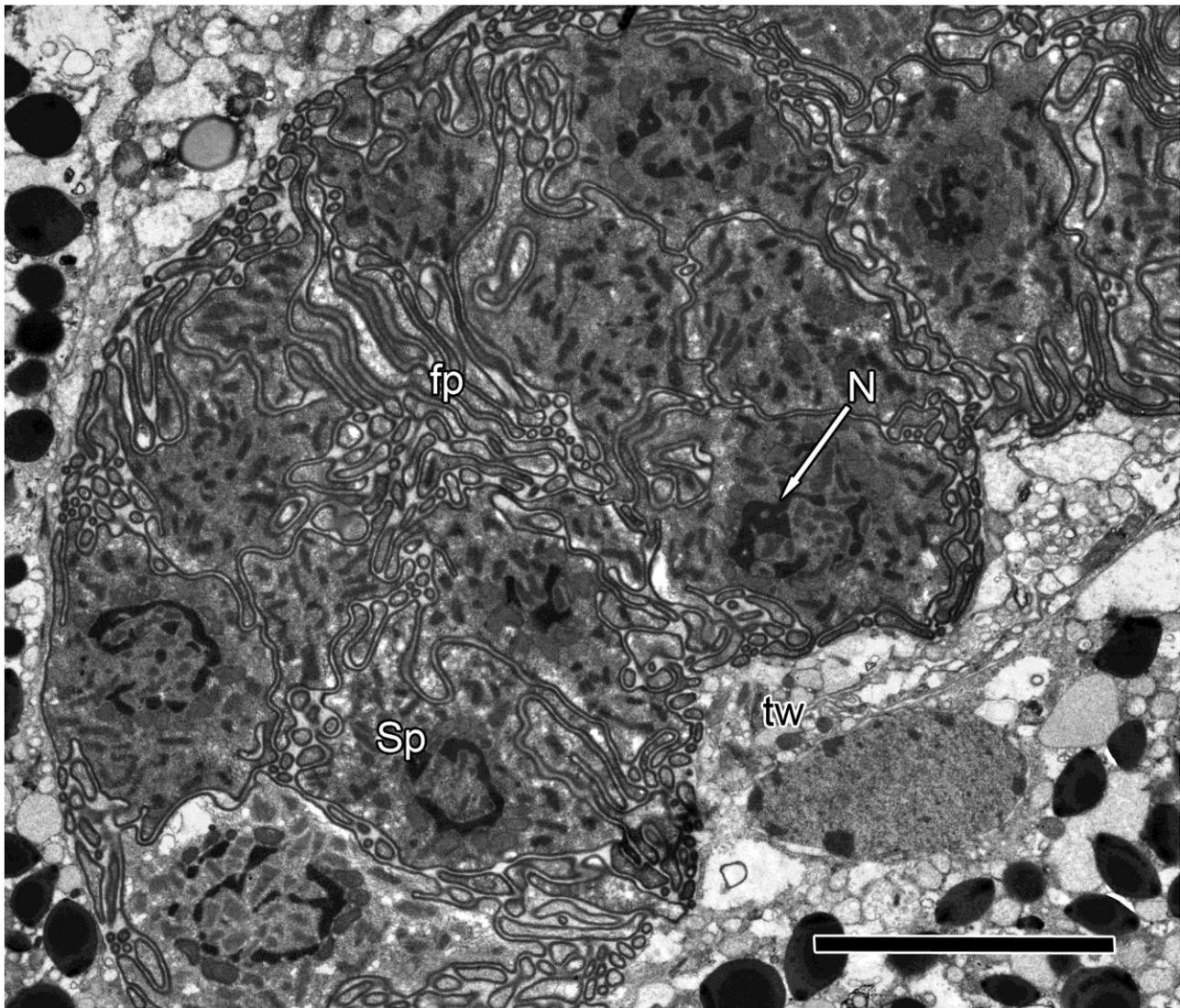
For transmission electron microscopy (TEM) the head and tail regions of each animal were cut off for facilitation of following tissue fixation and embedding. The specimens were fixed for TEM at 4°C in 2.5% glutaraldehyde in 0.05 M cacodylate buffer containing 21 mg ml<sup>-1</sup> NaCl overnight and then post-fixed 2 h in 1% osmium tetroxide in the same buffer containing 23 mg ml<sup>-1</sup> NaCl. Post-fixation was followed by *en bloc* staining for 2 h in 1% solution of uranyl acetate in distilled water; then the specimens were dehydrated in ethanol followed by isopropanol series and embedded in Epon resin. Embedded animals were cut longitudinally by glass knives to remove thick cuticle and obtain blocks, where internal tissues appear on semi-thin sections. The clay particles covering the cuticle surface, which make thin sectioning impossible, were dissolved by overnight incubation of blocks in 2% solution of hydrofluoric acid (HF) in distilled water with 10% acetone at room temperature. After washing in distilled water, the blocks were thoroughly dried overnight in an oven at 60°C.

Thin sections cut with a diamond knife using Leica UC6 ultratome were stained with lead citrate and examined with a JEOL JEM 100S and JEOL JEM 1010 transmission electron microscopes. The ultrastructure of spermatozoa filling the seminal

vesicle of the gravid female was studied. The spermatozoa from testis are termed as 'immature spermatozoa' following the basic terminology proposed by Shepherd (1981).



**Fig. 1.** *Desmoscolex granulatus*, female. A. Entire. B. Anterior body. C. Posterior body. Scale bars: A – 100  $\mu\text{m}$ ; B & C – 20  $\mu\text{m}$ .



**Fig. 2.** *Desmoscolex granulatus*, TEM. Longitudinal section through the testis with densely packed immature spermatozoa, general view at low magnification. Abbreviations: fp – filopodia; N – nucleus; Sp – spermatozoon; tw – testis wall. Scale bar: 5  $\mu$ m.

## RESULTS

**Taxonomy.** Since the species identification can be questionable without proper illustration, because of this new finding in a region remote from the type locality we consider it justifiable to provide a redescription.

### *Desmoscolex granulatus* Decraemer, 1975

**Material.** One adult female (Fig. 1).

**Locality.** North Vietnam, Tien Yen Estuary (21°18'997" N; 107°36'075" E). Silty sand, 0.5 m deep. April 2015.

**Description and measurements.** Body spindle-shaped, tapered to both ends and body cuticle

coarsely annulated. Body length 325  $\mu$ m,  $a = 5.9$ ,  $b = 5.08$ ,  $c = 5.0$ ,  $c' = 1.48$ . Main rings 18 in number, covered by broad desmen composed of large angular concretions making the body opaque. At mid-body, body diam. 55  $\mu$ m including desmen and 47  $\mu$ m without desmen. Inter-zones hardly discernible, also partly covered by smaller concretions.

Head rounded, wider than long (head length 15  $\mu$ m, width 22  $\mu$ m). Four cephalic setae rather long (16  $\mu$ m) and jointed, basal joint longer and stiff, distal joint short and thin (one fourth to third of the entire setae length). Amphideal fovea blister-like, wider than long (amphid length 7  $\mu$ m, width 13  $\mu$ m) and situated close to the cephalic apex.

Somatic subdorsal setae much longer than

subventral, jointed, basal joint long and stiff, distal joint short (about one third of the entire setae), lanceolate. Subventral setae short and smooth (not jointed). Length of subdorsal somatic setae: 1<sup>st</sup> – 20  $\mu\text{m}$ , 2<sup>nd</sup> – 16  $\mu\text{m}$ , 3<sup>d</sup> – 15  $\mu\text{m}$ , last but one – 20  $\mu\text{m}$ , terminal – 26  $\mu\text{m}$ . Length of 1<sup>st</sup> subventral somatic setae – 6.9  $\mu\text{m}$ .

All the somatic setae located in strictly bilateral pairs on the main rings. Position of subdorsal somatic setae on the main rings: 1, 3, 5, 7, 9, 11, 13, 17, 18 = 9. Position of subventral somatic setae on the main rings: 2, 4, 6, 8, 10, 12, 14, 16 = 8.

Yellow ocelli at the level of 2<sup>nd</sup> main ring. Pharynx not discernible.

Vulva not found. Internal reproductive organs not discernible.

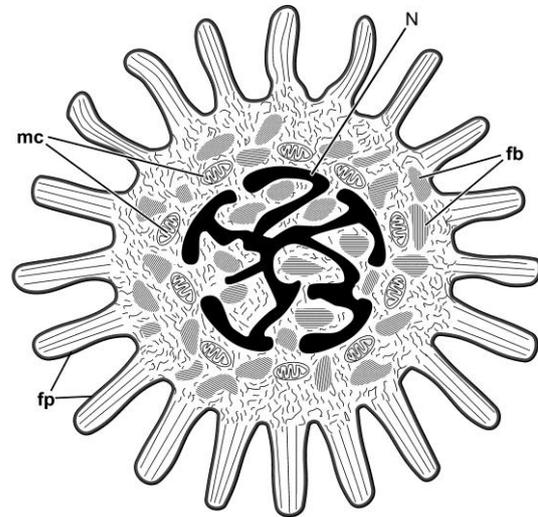
Anal tube 6  $\mu\text{m}$  long, covered with small concretions. Tail consists of two main rings. Terminal ring nearly rectangular and stout (length 41  $\mu\text{m}$ , basal width 21  $\mu\text{m}$ ). Terminal tube (spinneret) not developed. Posteriormost subdorsal setae attached at two thirds of the terminal ring close to its posterior end. Phasmata not observed.

**Remarks.** The species is characterised by body composed of 18 and tail of two main rings covered by desmen, vesicular amphideal fovea situated anteriorly on the head, jointed cephalic setae with thin distal part and jointed subdorsal somatic setae with lanceolate distal part. These features are shared with *D. granulatus* Decraemer, 1975 and *D. membranousus* Decraemer, 1974. Our specimen fits with both species descriptions in all dimensions and structures except for the presence of dark red-brownish granulation at the level of pharynx and anterior intestine, not observed in our specimens. Both *D. granulatus* and *D. membranousus* were found in the area of Great Barrier Reef at depths 21.5-35 m on sandy bottom or on sand covered with silt layer (Decraemer, 1974, 1975). Our specimens are designated as *D. granulatus* because of lack of any circumoral membrane specific for *D. membranousus*.

**Ultrastructure.** The testis of *D. granulatus* was filled with uniform germ cells identified as the immature spermatozoa. No previous developmental stages such as spermatids or spermatocytes were observed. Immature spermatozoa form a cluster of tightly packed cells surrounded by testis epithelium (Fig. 2). They have irregular amoeboid outlines and form numerous filopodia, which are squeezed between the spermatozoan bodies.

The spermatozoa have more or less uniform structure along the testis (Figs 2 & 3). They are unpolarized cells of average size *ca* 4-6  $\mu\text{m}$  with central nucleus (Figs 2 & 4A). On the thin sections

spermatozoan nuclei look like discrete dense particles but observations of many spermatozoa from the successive serial sections demonstrate that each nucleus is a highly lobated mass of strongly condensed nuclear chromatin with sharp boundaries devoid of a nuclear envelope (Figs 2; 4A & B).

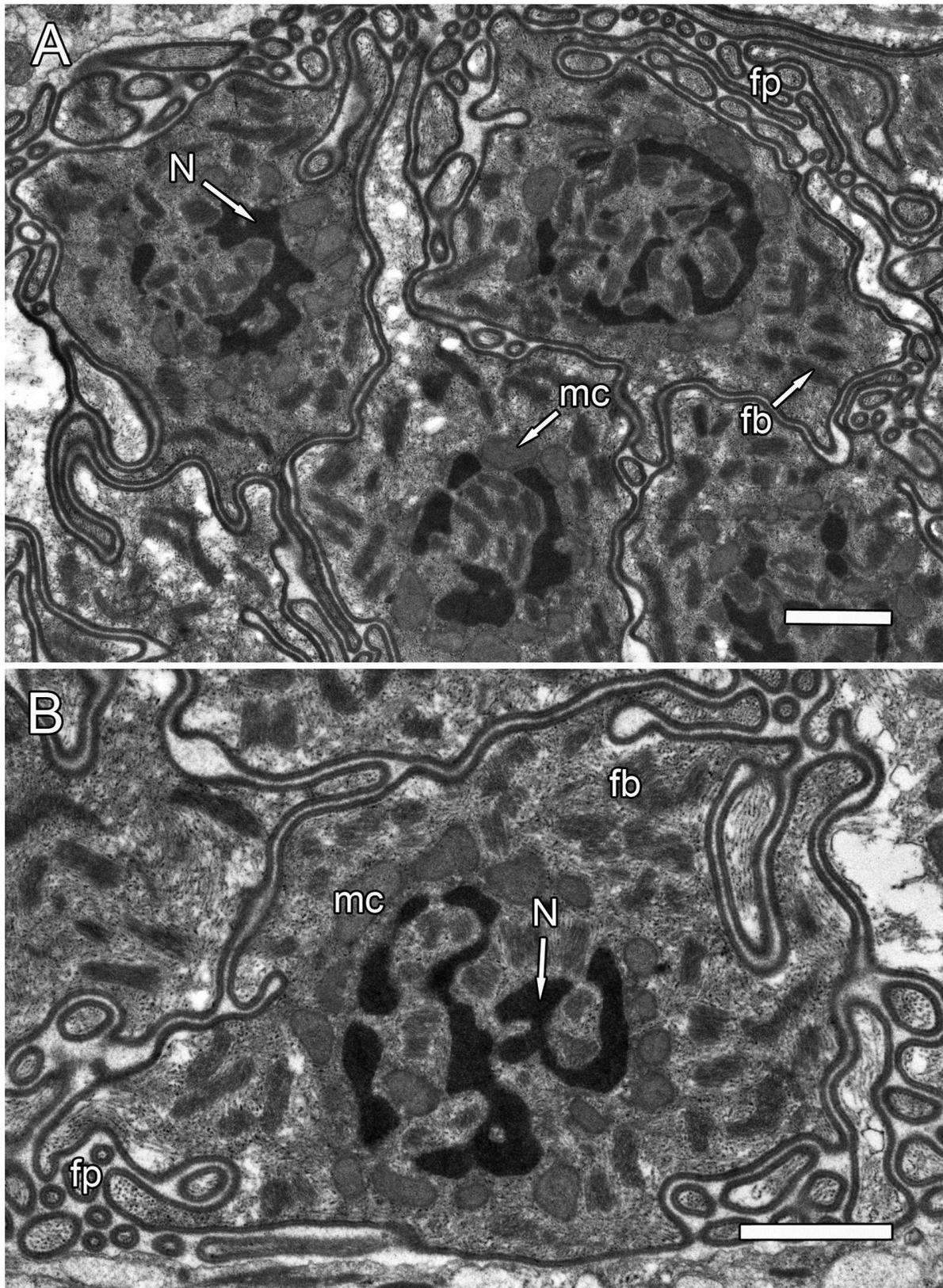


**Fig. 3.** *Desmoscolex granulatus*, schematic representation of the immature spermatozoan structure. Lobated nucleus (N) without nuclear envelope is surrounded by fibrous bodies (fb) and mitochondria (mc). The surface of the spermatozoan bears numerous filopodia (fp). Not to scale.

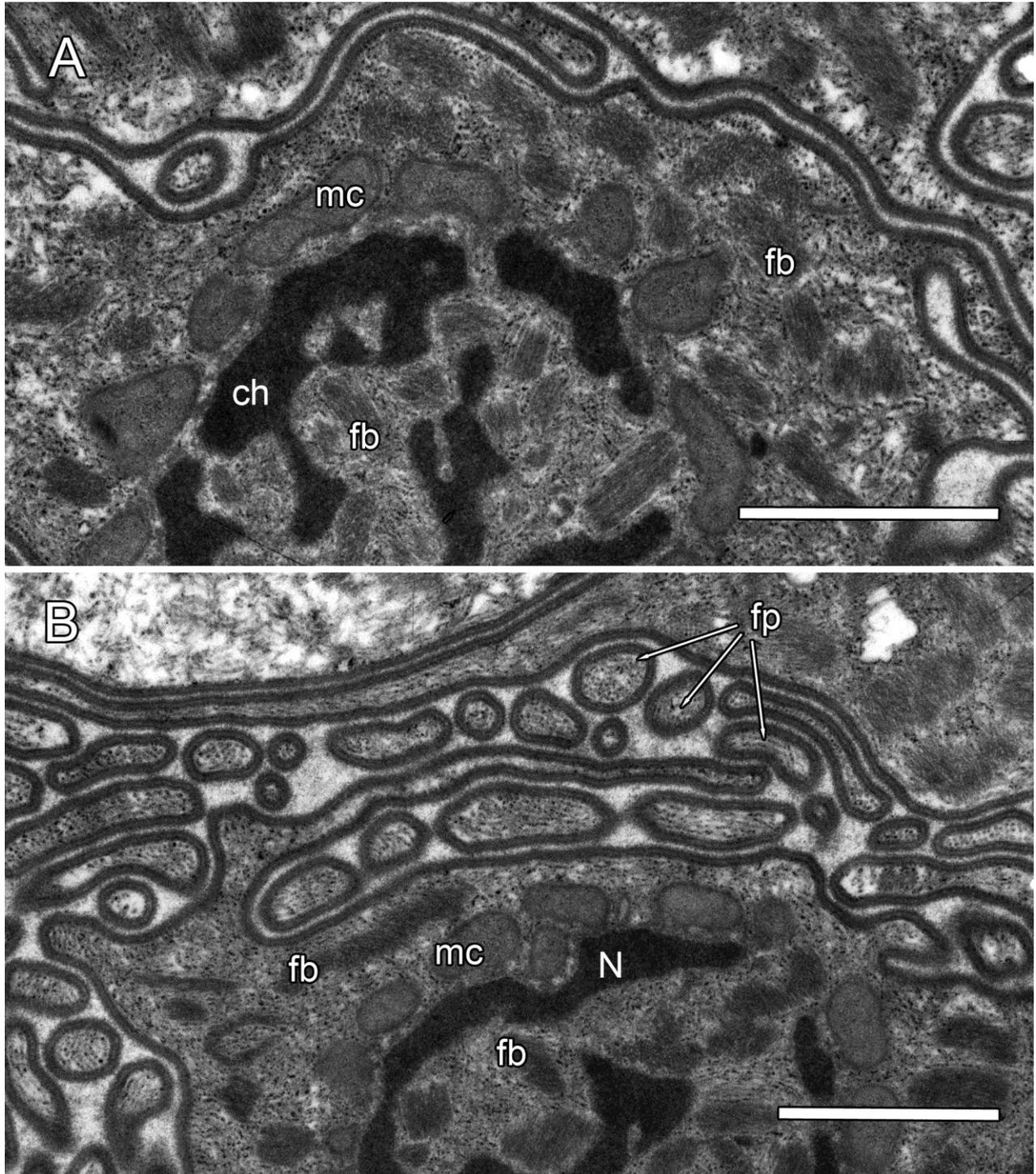
The cytoplasm of the immature spermatozoa contains only two types of components: mitochondria and bundles of filaments (Figs 4B; 5A & B). Elongated mitochondria (0.6  $\mu\text{m}$  long and 0.3  $\mu\text{m}$  wide) with opaque matrix surrounds the nucleus area (2-3  $\mu\text{m}$  in diam.) as a layer with narrow space between organelles and chromatin. Mitochondria appear rarely between nuclear lobes and are totally absent at the cell periphery (Fig. 4B).

Electron dense bundles of filaments found in spermatozoa of *D. granulatus* were similar to the fibrous bodies (FB) characteristic of spermatogenic cells of most nematodes studied. These FB are abundant, vary in size, and consist of tightly packed parallel fibres (Figs 5A, B & 6). The FB are evenly distributed throughout the cytoplasm of spermatozoa, they appear in-between the nuclear lobes and fill the cell periphery around the mitochondrial layer (Figs 4B & 5A).

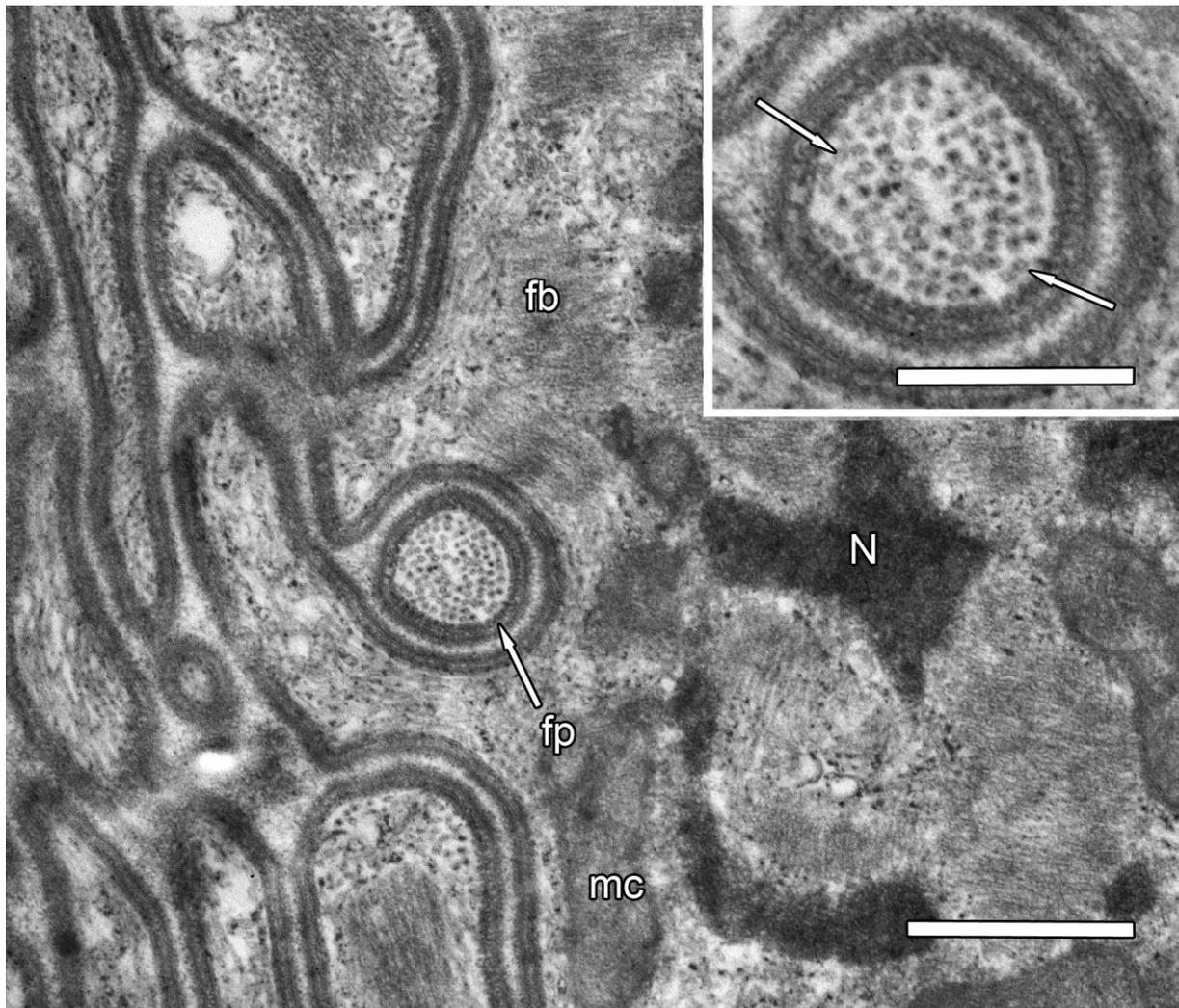
The cytoplasm of moderate density around the nucleus, mitochondria and FB is not homogenous but comprises apparently filamentous material containing parallel orientated fibres *ca* 15-18  $\mu\text{m}$  in diam. (Figs 5B & 6). The spermatozoan surface is organised into 0.24-0.32  $\mu\text{m}$  thick filopodia of variable



**Fig. 4.** Immature spermatozoa from testis of *Desmoscolex granulatus*, TEM. A. Cluster of spermatozoa. B. Immature spermatozoon, general view. fb – fibrous bodies; for other abbreviations see legend for Fig. 2. Scale bars: 1  $\mu$ m.



**Fig. 5.** Immature spermatozoa from testis of *Desmoscolex granulatus*, TEM. A. Central part and periphery of the spermatozoon at high magnification. B. Central part, periphery and filopodia of the spermatozoa. ch – chromatin; for other abbreviations see legend for Fig. 2. Scale bars: 1  $\mu$ m.



**Fig. 6.** Periphery of the immature spermatozoon of *Desmoscolex granulatus*, TEM, high magnification. Insert: enlargement of cross section through the filopodium showing tubule-like fibres (arrows) and complicated structure of cell wall. For abbreviations see legend for Fig. 2. Scale bars: 0.5  $\mu\text{m}$ ; insert – 0.25  $\mu\text{m}$ .

length and shape (Figs 2; 4A & 5B). The cytoplasm of filopodia is a continuation of the sperm cytoplasm and also contains characteristic parallel fibres (Figs 5B & 6). These fibres are strongly orientated parallel to a long axis of a filopodium forming well arranged fascicles apparent on cross sections through a filopodium (Fig. 6). On cross sections of filopodia and sections through the cell periphery fibres look like tubules (Fig. 6, insert).

The sperm cell membrane is covered by thin surface coat and reinforced from the inside with the thick dense internal layer. This 30 nm thick unit membrane complex looks like enormously thick envelope bordering of cell and filopodia (Figs 5B & 6).

No membranous organelles which are characteristic of most nematode spermatozoa were observed in immature spermatozoa of *D. granulatus*.

## DISCUSSION

The immature spermatozoa of *D. granulatus* have the basic ultrastructural features of the sperm cells of many nematodes studied so far: they lack an axoneme, an acrosome and a nuclear envelope (Justine & Jamieson, 1999; Justine, 2002). In general, these are unpolarized cells with a highly lobated nucleus surrounded by a layer of mitochondria and numerous fibrous bodies looking like bundles of filaments (Fig. 3). The numerous well developed filopodia are characteristic of spermatozoa.

Desmoscolecida is a well defined group of nematodes considered by most authors since Filipjev (1929) as a separate order or sometimes as a suborder within the chromadorean clade of Nematoda. Lorenzen (1981) in the first German

edition of his influential book put desmoscolecids (as Desmoscolecoida) in the Monhysterida because of the outstretched ovaries. In the second English edition (Lorenzen, 1994) Desmoscolecina are placed by him in Chromadorida based on the opinion that outstretched ovaries might have developed from antidiromously reflexed ovaries. According to analysis of nucleotide sequences of 18S rDNA (Hwang *et al.*, 2009) of species from four desmoscolecid genera, Desmoscolecida forms a monophyletic group positioned as a sister group of the clade including members of Monhysterida and Araeolaimida (both orders are characterised morphologically by outstretched ovaries). Now the order Desmoscolecida is placed within the subclass Chromadorea of the class Chromadorea (De Ley & Blaxter, 2004; Hodda, 2007). From the morphological point of view, the position of Desmoscolecida within class Chromadorea and subclass Chromadorea is completely justified but the relationships of Desmoscolecida to either Monhysterida or Chromadorida, or Plectida is still subject to debate (Decraemer & Rho, 2014). What can sperm structure say on this topic?

The main pattern of spermatogenesis in the nematode class Chromadorea is marked by development of specific organelles, MO and FB as the FB-MO complexes (Yushin & Malakhov, 2004, 2014) This 'rhabditid pattern' was described for a variety of representatives of the order Rhabditida (Spiruromorpha, Ascaridomorpha, Panagrolaimomorpha, Tylenchomorpha, Diplogasteromorpha, Rhabditomorpha, and Myolaimina), as well as for the aquatic nematodes of the orders Monhysterida (Monhysteroidea), Araeolaimida and Plectida (Justine & Jamieson, 1999; Justine, 2002; Giblin-Davis *et al.*, 2010; Yushin & Malakhov, 2004, 2014; Zograf, 2014; Slos *et al.*, 2015; Limantseva *et al.*, 2015).

The second pattern is characterised by absence of MO, while free FB are well developed and possibly have the same nature as the FB of other nematodes. This pattern was described in some Rhabditida and free-living marine nematodes from the orders Chromadorida, Desmodorida and Monhysterida (Justine & Jamieson, 1999; Justine, 2002; Yushin & Malakhov, 2004, 2014). In some cases of simplification complete reduction of aberrant sperm components have also been observed (Justine, 2002; Yushin & Malakhov, 2004, 2014).

Spermatozoa of *D. granulatus* having FB but devoid of MO fit with the second pattern of spermatozoon structure of Chromadorea, which was described in three families of the order Chromadorida – Chromadoridae (*Neochromadora poecilosoma*), Cyatholaimidae (*Paracyatholaimus*

*pugettensis*), Selachinematidae (*Halichoanolaimus* spp.) as well as in the order Desmodorida (Desmodoridae, *Metachromadora itoi*), Monhysterida (Linhomoeidae, *Paralinhomoeus* sp., *Terschellingia glabricutis*) and Rhabditida (Tylenchoidea) (Yushin & Coomans, 2000, 2005; Justine, 2002; Yushin & Zograf, 2002, 2004; Zograf & Yushin, 2004; Zograf *et al.*, 2004; Yushin, 2003, 2007, 2008; Yushin & Malakhov, 2014). Unlike *D. granulatus*, the FB in immature spermatozoa of these nematodes look more developed, appearing as large amorphous or paracrystalline bodies.

Numerous well developed filopodia are another distinct morphological feature of the immature spermatozoa of *D. granulatus*. Filopodia have been observed in spermatogenic cells of many nematodes from distant taxa of nematodes from both classes (Riemann, 1983; Justine & Jamieson, 1999; Justine, 2002; Yushin & Zograf, 2004; Zograf *et al.*, 2004; 2008; Zograf & Yushin, 2004; Yushin, 2003, 2007, 2008, 2010; Lak *et al.*, 2015; Yushin *et al.*, 2016). Observation of *D. granulatus* spermatozoa confirms the wide distribution of filopodia and their importance in development and physiology of nematode male gametes.

The cytoplasm of immature spermatozoa of *D. granulatus* contains characteristic tubule-like fibres arranging as a fascicle inside filopodia. Similar fibres have been observed earlier in the spermatozoa of many nematodes representing very distant taxa from both classes of the phylum, Enoplea and Chromadorea (Beams & Sekhon, 1972; Shepherd *et al.*, 1973; Baccetti *et al.*, 1983; Shepherd & Clark, 1983; Hess & Poinar, 1989; Poinar & Hess-Poinar, 1993; Cares & Baldwin, 1994, 1995; Takahashi *et al.*, 1994; Endo *et al.*, 1998; Turpeenniemi, 1998; Yushin, 2004, 2007, 2008, 2010; Yushin & Zograf, 2004; Zograf *et al.*, 2004). These fibres (microtubule-like fibres, MLF) resemble the cytoskeleton microtubules of Metazoa, but they have a diameter 13 to 20 nm (15-18 nm in *D. granulatus*) and cannot be identified as classic tubulin-containing microtubules, which have a normal diameter of 24-25 nm (Stephens & Edds, 1976). Moreover, it was shown unequivocally that microtubules and tubulin are absent in nematode spermatozoa, except the centrioles (if present) and their derivatives (Mansir & Justine, 1998). The prevalent cytoskeleton protein MSP is the base for cell structure and movement (Justine, 2002; Yushin *et al.*, 2016). It is likely that the MLF in spermatozoa of *D. granulatus* and other nematodes are assembled from the MSP-based filaments. The MLF fascicles apparently serve as an axial skeleton for the sperm filopodia of *D. granulatus*.

The spermatozoon pattern with occurrence of the FB and absence of MO unites *D. granulatus* with some chromadorids, desmodorids, monhysterids and rhabditids (Tylenchoidea). This conclusion is supported by the filopodial nature of the sperm surface and abundance of MLF demonstrated by these taxa.

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## REFERENCES

- BACCETTI, B. 1985. Evolution of the sperm cell. In: *Biology of Fertilization, Volume 2: Biology of the Sperm* (C.B. Metz & A. Monroy Eds). pp. 3-58. Orlando, USA, Academic Press.
- BACCETTI, B., DALLAI, R., GRIMALDI DE ZIO, S. & MARINARI, A. 1983. The evolution of the nematode spermatozoon. *Gamete Research* 8: 309-323.
- BEAMS, H.W. & SEKHON, S.S. 1972. Cytodifferentiation during spermatogenesis in *Rhabditis pellio*. *Journal of Ultrastructure Research* 38: 511-527.
- CARES, J.E. & BALDWIN, J.G. 1994. Comparative fine structure of sperm of *Verutus volvingentis* and *Meloidodera floridensis* (Heteroderinae, Nematoda). *Canadian Journal of Zoology* 72: 1481-1491.
- CARES, J.E. & BALDWIN, J.G. 1995. Comparative fine structure of sperm *Heterodera schachtii* and *Punctodera chaltoensis*, with phylogenetic implications for Heteroderinae (Nemata: Heteroderidae). *Canadian Journal of Zoology* 73: 309-320.
- CHU, D.S. & SHAKES, D.C. 2013. Spermatogenesis. In: *Germ Cell Development in C. elegans. Advances in Experimental Medicine and Biology, Volume 757* (T. Schedl Ed.). pp. 171-203. New York, USA, Springer Science + Business Media B.V.
- CLAPARÈDE, E. 1863. *Beobachtungen über die Anatomie und Entwicklungsgeschichte wirbelloser Tiere an der Küste der Normandie angestellt*. Germany, Wilhelm Engelmann. 120 pp.
- DALLAI, R., GOTTARDO, M. & BEUTEL, R.G. 2016. Structure and evolution of insect sperm: new interpretations in the age of phylogenomics. *Annual Review of Entomology* 61: 1-23.
- DECRAEMER, W. 1974. Scientific report on the Belgian expedition to the Great Barrier Reef in 1967. Nematodes V: observations on *Desmoscolex* (Nematoda, Desmoscolecida) with descriptions of three new species. *Zoologica Scripta* 3: 243-255.
- DECRAEMER, W. 1975. Scientific report on the Belgian expedition to the Great Barrier Reef in 1967. Nematodes I: *Desmoscolex*-species (Nematoda – Desmoscolecida) from Yonge Reef, Lizard Island and Nympe Island with general characteristics of the genus *Desmoscolex*. *Annales de la Société Royale Zoologique de Belgique* 104: 105-130.
- DECRAEMER, W. & RHO, H.S. 2014. Order Desmoscolecida. In: *Handbook of Zoology. Gastrotricha, Cycloneuralia and Gnatifera. Volume 2: Nematoda* (A. Schmidt-Rhaesa Ed.). pp. 351-372. Berlin/Boston, Germany/USA, Walter de Gruyter GmbH.
- DE GRISSE, A.T. 1969. Redescription ou modifications de quelques techniques utilisées dans l'étude des nématodes phytoparasitaires. *Mededelingen van de Rijksfakulteit Landbouwwetenschappen Gent* 34: 351-369.
- DE LEY, P. & BLAXTER, M. 2002. Systematic Position and Phylogeny. In: *The Biology of Nematodes* (D.L. Lee Ed.). pp. 1-30. London, UK, Taylor & Francis Group.
- DE LEY, P. & BLAXTER, M. 2004. A new system for Nematoda: combining morphological characters with molecular trees, and translating clades into ranks and taxa. In: *Nematology Monographs and Perspectives, Volume 2* (R. Cook & D.J. Hunt Eds). pp. 633-653. Leiden, The Netherlands, E.J. Brill.
- ENDO, B.Y., ZUNKE, U & WERGIN, W.P. 1998. Spermatogenesis in the lesion nematode, *Pratylenchus penetrans* (Nemata, Pratylenchidae). *Journal of the Helminthological Society of Washington* 65: 227-242.
- FILIPJEV, I.N. 1929. Classification of freeliving Nematoda and relations to parasitic forms. *Journal of Parasitology* 15: 281-282.
- GIBLIN-DAVIS, R.M., KANZAKI, N., DE LEY, P., WILLIAMS, D.S., SCHIERENBERG, E., RAGSDALE, E.J., ZENG, Y. & CENTER, B.J. 2010. Ultrastructure and life

- history of *Myolaimus byersi* n. sp. (Myolaimina: Myolaimidae) a phoretic associate of the crane fly, *Limonia schwarzi* (Alexander) (Limoniidae), in Florida. *Nematology* 12: 519-542.
- HESS, R. & POINAR, G.O., JR. 1989. Sperm development in the nematode *Neoaplectana intermedia* (Steinernematidae: Rhabditida). *Journal of Submicroscopical Cytology and Pathology* 21: 543-555.
- HODDA, M. 2007. Phylum Nematoda. *Zootaxa* 1668: 265-293.
- HWANG, U.W., CHOI, E.H., KIM, D.S., DECRAEMER, W. & CHANG, C.Y. 2009. Monophyly of the family Desmoscolecidae (Nematoda, Desmoscolecida) and its phylogenetic position inferred from 18S rDNA sequences. *Molecules and Cells* 27: 515-523.
- JAMIESON, B.G.M., AUSIO, J & JUSTINE, J.-L. 1995. Advances in spermatozoal phylogeny and taxonomy. *Mémoires du Muséum National d'Histoire Naturelle* 166: 119-128.
- JUSTINE, J.-L. 2002. Male and female gametes and fertilization. In: *The Biology of Nematodes* (D.L. Lee Ed.). pp. 73-119. London, UK, Taylor & Francis Group.
- JUSTINE, J.-L. & JAMIESON, B.G.M. 1999. Nematoda. In: *Reproductive Biology of Invertebrates, Volume IX, part B* (B.G.M. Jamieson Ed.). pp. 183-266. New Delhi, India, Oxford & IBH Publishing.
- LAK, B., YUSHIN, V.V., SLOS, D., CLAYES, M., DECRAEMER, F. & BERT, W. 2015. High-pressure freezing and freeze-substitution fixation reveal the ultrastructure of immature and mature spermatozoa of the plant-parasitic nematode *Trichodorus similis* (Nematoda; Triplonchida; Trichodoridae). *Micron* 77: 25-31.
- LEVRON, C., MIQUEL, J., OROS, M. & SCHOLZ, T. 2010. Spermatozoa of tapeworms (Platyhelminthes, Eucestoda): advances in ultrastructural and phylogenetic studies. *Biological Reviews of the Cambridge Philosophical Society* 85: 523-543.
- LIANA, M. & WITALINSKI, W. 2005. Sperm structure and phylogeny of Astigmata. *Journal of Morphology* 265: 318-324.
- LIMANTSEVA, L.A., YUSHIN, V.V., BERT, W., CLAEYS, M. & SLOS, D. 2015. Spermatogenesis and sperm ultrastructure of *Anaplectus porosus* Allen & Nofsinger, 1968 (Nematoda: Plectida). *Russian Journal of Nematology* 23: 162.
- LORENZEN, S. 1981. Entwurf eines phylogenetischen system der freilebenden Nematoden. *Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven* 7: 1-432.
- LORENZEN, S. 1994. *The Phylogenetic Systematics of Free-Living Nematodes*. UK, the Ray Society, 383 pp.
- MANSIR, A & JUSTINE, J.-L. 1998. The microtubular system and posttranslationally modified tubulin during spermatogenesis in a parasitic nematode with ameboid and aflagellate spermatozoa. *Molecular Reproduction and Development* 49: 150-167.
- PITNICK, S., HOSKEN, D.J. & BIRKHEAD, T.R. 2009. Sperm morphological diversity. In: *Sperm Biology: an Evolutionary Perspective* (T.R. Birkhead, D.J. Hosken & S. Pitnick Eds). pp. 69-149. San Diego, USA, Academic Press.
- POINAR, G.O. JR. & HESS-POINAR, R.T. 1993. The fine structure of *Gastromermis* sp. (Nematoda: Mermithidae) sperm. *Journal of Submicroscopic Cytology and Pathology* 25: 417-431.
- RIEMANN, F. 1983. Observations on spermatozoa in aquatic nematodes. *Systematics Association (Special Volume)* 22: 85-93.
- RIEMANN, F. & RIEMANN, O. 2010. The enigmatic mineral particle accumulations on the cuticular rings of marine desmoscolecoid nematodes – structure and significance explained with clues from live observations. *Meiofauna Marina* 18: 1-10.
- SEINHORST, J.W. 1959. A rapid method for the transfer of nematodes from fixative of anhydrous glycerine. *Nematologica* 4: 67-69.
- SHEPHERD, A.M. 1981. Interpretation of sperm development in nematodes. *Nematologica* 27: 122-125.
- SHEPHERD, A.M. & CLARK, S.A. 1983. Spermatogenesis and sperm structure in some *Meloidogyne* species (Heterodera, Heteroderidae). *Revue de Nématologie* 6: 17-32.
- SHEPHERD, A.M., CLARK, S.A. & KEMTON, A. 1973. Spermatogenesis and sperm ultrastructure in some cyst nematodes, *Heterodera* spp. *Nematologica* 19: 551-560.
- SLOS, D., ENSAFI, P., CLAEYS, M., YUSHIN, V.V., DECRAEMER, W. & BERT, W. 2015. Ultrastructure of sperm development in the genus *Ditylenchus* (Nematoda: Anguinidae). *Nematology* 17: 313-324.
- STEPHENS, R.E. & EDDS, K.T. 1976. Microtubules: structure, chemistry, and function. *Physiological Reviews* 56: 709-777.
- TAKAHASHI, Y., GOTO, C. & KITA, K.K. 1994. Ultrastructural study of *Trichinella spiralis* with emphasis on adult male reproductive organs. *Journal of Helminthology* 68: 353-358.
- TIMM, R.W. 1970. A revision of the nematode order Desmoscolecida Filipjev, 1929. *University of California Publications in Zoology* 93: 1-115.
- TURPEENIEMI, T.A. 1998. Ultrastructure of spermatozoa in nematode *Halalaimus dimorphus* (Nemata: Oxystominidae). *Journal of Nematology* 30: 391-403.
- YUSHIN, V.V. 2003. Ultrastructure of spermatozoa in the free-living marine nematode of the family Selachinematidae (Chromadorida: Cyatholaimina). *Russian Journal of Nematology* 11: 81-90.

- YUSHIN, V.V. 2004. New data on sperm structure in mononchid nematodes (Enoplia, Mononchida). *Russian Journal of Nematology* 12: 139-142.
- YUSHIN, V.V. 2007. Spermatogenesis in the free-living marine nematode of the family Linhomoeidae (Nematoda, Monchysterida). *Russian Journal of Nematology* 14: 109-116.
- YUSHIN, V.V. 2008. Sperm dimorphism in the free-living marine nematode *Terschellingia glabricutis* (Nematoda: Monhysterida: Linhomoeidae). *Nematology* 10: 189-205.
- YUSHIN, V.V. 2010. Spermatid and spermatozoon structure in fresh-water nematode *Paractinolaimus microdentatus* (Nematoda: Dorylaimida: Actinolaimidae). *Russian Journal of Nematology* 18: 199-208.
- YUSHIN, V.V., CLAEYS, M. & BERT, W. 2016. Ultrastructural immunogold localization of major sperm protein (MSP) in spermatogenic cells of the nematode *Acrobeles complexus* (Nematoda, Rhabditida). *Micron* 89: 43-55.
- YUSHIN, V.V. & COOMANS, A. 2000. Ultrastructure of sperm development in the free-living marine nematodes of the family Chromadoridae (Chromadorida: Chromadorina). *Nematology* 2: 285-296.
- YUSHIN, V.V. & COOMANS, A. 2005. Ultrastructure of sperm development in the free-living marine nematode *Metachromadora itoi* (Chromadorida, Desmodorida). *Acta Zoologica* 86: 255-265.
- YUSHIN, V.V. & MALAKHOV, V.V. 2004. Spermatogenesis and nematode phylogeny. In: *Proceeding of the Fourth International Congress of Nematology (Tenerife, Spain, 2002). Nematology Monographs and Perspectives, Volume 2* (R. Cook & D.J. Hunt Eds). pp. 655-665. Leiden, The Netherlands, E.J. Brill.
- YUSHIN, V.V. & MALAKHOV, V.V. 2014. The origin of nematode sperm: Progenesis at the cellular level. *Russian Journal of Marine Biology* 40: 71-81.
- YUSHIN, V.V. & SPIRIDONOV, S.E. 2001. Ultrastructure of sperm development in *Heth mauriesi* Adamson, 1982 (Rhigonematida: Hethidae). *Russian Journal of Nematology* 9: 119-126.
- YUSHIN, V.V. & ZOGRAF, J.K. 2002. Electron-microscopic study of spermatogenesis of free-living marine nematodes *Neochromadora poecilosoma* (Nematoda, Chromadorida). *Russian Journal of Marine Biology* 2: 37-42.
- YUSHIN, V.V. & ZOGRAF, J.K. 2004. Ultrastructure of spermatozoa in free-living marine nematode *Paracanthonchus macrodon* (Nematoda, Chromadorida). *Invertebrate Reproduction and Development* 45: 59-67.
- ZOGRAF, J.K. 2014. Ultrastructure of spermatogenesis and sperm of the free-living soil nematode *Panagrellus redivivus* (Rhabditida: Panagrolaimidae). *Russian Journal of Nematology* 22: 39-48.
- ZOGRAF, J.K. & YUSHIN, V.V. 2004. Ultrastructural study of spermatogenesis in the free-living marine nematode *Paracyatholaimus pugettensis* Wieser et Hopper, 1967 (Chromadorida: Cyatholaimidae). *Russian Journal of Marine Biology* 30: 395-401.
- ZOGRAF, J.K., YUSHIN, V.V. & MALAKHOV, V.V. 2004. Ultrastructure of spermatogenesis in the free-living nematode *Halichoanolaimus sonorus* (Chromadorida, Selachinematidae). *Nematology* 6: 800-807.
- ZOGRAF, J.K., ASTAKHOVA, A.A. & YUSHIN, V.V. 2008. Ultrastructure of spermatozoa in the free-living marine nematode *Monoposthia costata* (Chromadorida, Desmodorida). *Russian Journal of Nematology* 16: 121-128.

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**Zograf, J. K., Nguyen Dinh Tu, Nguyen Thi Xuan Phuong, Cao Van Luong, A. V. Tchesunov, V. V. Yushin.** Первое ультраструктурное исследование сперматозоидов десмосколецид (Nematoda: Desmoscolecida: Desmoscolecidae).

**Резюме.** Проведено электронно-микроскопическое изучение морских нематод *Desmoscolex granulatus* (Desmoscolecida). Сперматозоид представляет собой неполяризованную клетку с многочисленными филоподиями, с центральным многолопастным ядром, лишенным ядерной оболочки. В цитоплазме имеются митохондрии и фиброзные тела (FB). В сперматозоидах *D. granulatus* отсутствуют мембранные органеллы (МО), характерные для многих нематод. По особенностям строения сперматозоидов, в которых FB не ассоциировано с МО, *D. granulatus* близок к некоторым хроматоридам, десмодоридам (Desmodoridae), монхистеридам (Linhomoeidae) и тиленхоморфным нематодам (Tylenchoidea). В пользу такого заключения говорит и структура филоподий этих нематод.

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